On the Eye-spot and Flagellum in *Euglena viridis*.

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(PLATE 32.)

EUGLENA VIRIDIS is commonly found in stagnant water which contains a considerable amount of organic matter. It is often found abundantly in the liquid which runs from manure heaps, forming at certain periods a dense green scum on its surface. It is found sometimes in water troughs, and on mud in roadside gutters in places where refuse water from houses is thrown; and it is often very abundant on sewage farms.

Under ordinary conditions it is a free swimming organism, capable of moving very rapidly through the water by means of a cilium, or flagellum, which is placed at the anterior end of the body; but it can exist for a long time in a non-motile condition in an encysted state, surrounded and protected by a very thick cell-membrane. It is, however, capable of passing again into the motile condition as soon as the environment becomes favourable.

General Structure.

An excellent account of the general structure and life-history of the genus *Euglena* is given by Klebs *, whose memoir records the literature on the subject up to the date of its publication. A large amount of information concerning the structure of the various species of *Euglena* and allied genera is given by Stein †, Bütschli ‡, Saville Kent §; and, more recently, a good general account of the group will be found in the first volume of the

* "Ueber die Organisation einiger Flagellaten-Gruppen und ihre Beziehungen zu Algen und Infusorien." Unters. aus dem bot. Inst. zu Tübingen, Bd. I., 1881–85.

† Der Organismus der Infusionsthiere, III. Der Organismus der Flagellaten. Leipzig, 1878.

‡ Bronn's Classen und Ordnungen des Thierreichs, I. Protozoa, 1883–87.

§ A Manual of the Infusoria. London, 1880–82.

'Traité de Zoologie Concrète' by Yves Delage and Edgard Hérouard *.

A memoir by Khawkine † also contains valuable information on the structure and physiology of *Euglena viridis*. For further information concerning the numerous memoirs in which the structure of *Euglena* is dealt with, the reader is referred to the papers quoted above, which contain complete bibliographical lists of the literature on the subject.

Without attempting to give a complete account of the structure of *Euglena viridis*, it may be useful to briefly summarize what is contained on the subject in the memoirs already published, which my own observations enable me to confirm.

In the free swimming condition, the animal is elongate and cylindrical in shape, slightly larger in the middle than at the ends; the anterior end being truncated, the posterior usually pointed. It is a unicellular organism, protected on the outside by a thin skin or layer of modified protoplasm which is striated obliquely by slightly elevated ridges. These can be made visible by crushing the cell and squeezing out the protoplasmic contents under a cover-glass. Under certain conditions, of which one appears to be malnutrition, I find that the cells, while still retaining their power of movement, become curiously distorted and deformed, and might easily be mistaken for distinct species. This apparently accompanies a process of slow disintegration.

The protoplasm contains numerous chlorophyll-bodies, sometimes scattered all over the cell, with the exception of a short space at the anterior end which always remains colourless, but more often radiating from the centre, leaving both anterior and posterior ends free. In many cases, especially in cells freshly collected, it is not easy to distinguish the separate chlorophyll-bodies; and this led Saville Kent (*loc. cit.*) to regard the chlorophyll as diffused through the protoplasm; but it is only necessary to keep such cells in obscurity for a short time, in ordinary tap-water, or to examine them under a high power of the microscope, to see that the chlorophyll-bodies are really definite organs of the cell ‡.

* Vol. I. La Cellule et les Protozoaires. Paris, 1896.

† "Recherches biologiques sur l'*Astasia ocellata* n. s. et l'*Euglena viridis*." Ann. des Sci. Nat. 7th series, vol. i., 1886.

‡ Klebs, *l. c.* p. 264. See also Jessie A. Sallitt, "On the Chlorophyll Corpuscles of some Infusoria," Q. J. M. S. 1884.

I find that the chlorophyll-bodies are capable of assuming different shapes—spherical, oval, elongate, and ribbon-like; and to this may be due the different views as to their nature taken by various observers.

The cell usually contains a large number of grains of a starch-like composition (*paramylum*), which are not coloured blue by iodine, and are therefore not pure starch. They possess a very definite and characteristic structure, which is correctly figured by Klebs *, presenting some resemblance to the structure of a typical starch grain in being composed of laminæ, but differing from the starch grain in this, that the laminæ are not concentric, and present the appearance of flat plates laid one upon the other.

These paramylum grains may be found in any part of the cell, but under normal conditions are generally more numerous in the region of the chlorophyll-bodies. In cells kept in the dark, in a good nutrient solution such as a dilute solution of potato-starch †, the chlorophyll-bodies become pushed to the posterior end of the cell, whilst the anterior two-thirds of the cell become filled with a mass of paramylum grains. This results in the production of the so-called "white" or "colourless" forms.

The nucleus is usually found at the posterior end of the cell, but it may occur in the middle, or very rarely at the anterior end; in the "white" forms just described, it is found between the chlorophyll-bodies and the paramylum grains. In normal cells the nucleus is posterior to the chlorophyll-bodies; in the "white" forms it is anterior to them. It can be very easily seen in the living cell, and with care its structure can be made out. In osmic acid, and other hardening and preserving fluids, the structure is brought out very clearly. It consists of a central spherical nucleolus surrounded by a granular network.

The anterior end of the cell has a depression leading into a narrow tube or gullet, out of which the flagellum passes. On the dorsal side of the gullet is a distinct eye-spot, and near it is a large clear space, called the principal vacuole; and on one side of this a single, pulsating vacuole.

* *Loc. cit.* See plate ii. figs. 8 *a*, 8 *b*, 8 *c*.

† Khawkine, *l. c.*

The Vacuole System and Gullet.

At the anterior, colourless end of all species of *Euglena* and allied genera there is a sharply defined space, which was known to Ehrenberg, and has been described by Klebs * as the "principal vacuole," into which open one or more pulsating vacuoles †. In *Euglena viridis* there is only one pulsating vacuole, but in other species there may be two or more, and in some cases the single pulsating vacuole is formed by the fusion of several smaller ones ‡. The principal vacuole possesses the power of slowly contracting, by which the liquid contained in it is discharged; but it is not to be regarded as a true contractile vacuole, but rather as a reservoir for liquid; and as a part of this liquid is poured into it by the true pulsating vacuoles, it would probably be better to describe it as an "excretory reservoir." The gullet is a conical or tube-like depression in the blunt anterior end of the cell, and it is usually described and figured as terminating in the neighbourhood of the excretory reservoir, from which it is separated by a thin layer of protoplasm only.

According to the view at present held, the excretory reservoir discharges its contents into the gullet through an opening which is made, at the moment when the contraction begins, by the rupture of the thin layer of protoplasm which separates the reservoir from the gullet §. Klebs ||, however, states that no opening can be observed in the excretory reservoir; it always remains sharply defined, and its slow contraction shows that such an opening is improbable.

I found, however, upon a careful examination of *Euglena viridis*, that, contrary to the observations described above, there is a permanent communication between the excretory reservoir and the gullet. This is not easy to observe in the living cells, but is very clearly seen when they are placed in a half to one per cent. solution of osmic acid for several hours, or even days, then mounted in dilute glycerine and observed under a $\frac{1}{12}$th inch oil-immersion objective, with the aid of a good sub-stage condenser.

* *Loc. cit.* p. 246.

† Carter, "Additional Notes on the Freshwater Infusoria in the Island of Bombay," Ann. & Mag. Nat. Hist. ser. 2, vol. xx. p. 34. Stein, *l. c.*

‡ Klebs, *l. c.*

§ Traité Zool. Concrète, i. p. 346.

|| *Loc. cit.* p. 248.

It is then seen that the external opening of the depression at the anterior end of the cell is slightly funnel-shaped, that it leads into the gullet, a narrow tube-like passage, which is curved towards the dorsal side of the animal, and opens at its distal end into the so-called principal vacuole or excretory reservoir (Pl. 32. figs. 2–8).

The cavity of the excretory reservoir is thus in communication with the exterior; and this affords a more satisfactory explanation of the discharge of the liquid poured into it by the pulsating vacuole than that given by previous observers.

This connection between the excretory reservoir and the gullet does not appear to have been recognized by previous observers; although Stein (*l. c.*) figures what appears to me to be a distinct connection between them in *Euglena deses*, Ehrbg., *E. Spirogyra* and *Colacium calvium*, Stein.

As to the function of this anterior cavity we know very little. The flagellum arises in it, and it is often stated that the ingestion of solid food material can take place through it, small particles of solid matter being forced down into the protoplasm at the base of the opening by the continual movement of the flagellum, where they become digested in the same way as in *Amœba* *.

But it is doubtful whether this can be definitely proved, and the evidence at present brought forward in favour of it is not altogether satisfactory. I have constantly repeated the experiment, which is commonly taken as a demonstration that *Euglena* possesses the power of absorbing solid food-material, of putting finely powdered carmine in water containing *Euglena*-cells in active movement; and have observed that the motion of the flagellum causes a rapid and violent movement of the carmine grains, often sweeping them up to and past the mouth-opening in large numbers; but I have never been able to satisfy myself that any of them ever get into the gullet. Dangeard † states that the nutrition in the whole group is distinctly vegetal in character; and that no solid particles penetrate the interior of the body. The gullet, according to him, certainly does not serve for the introduction of solid matter.

Khawkine (*l. c.*) suggests that liquid nutriment may be

* See, for example, Parker's 'Elements of Biology,' p. 40.

† "Recherches sur les Cryptomonadinæ et les Euglenæ." Le Botaniste, i. 1889, p. 1.

absorbed through the gullet, and he brings forward the following observation in support of this view. When *Euglenæ* are kept in dilute potato-starch solution in the dark, paramylum grains are produced in great abundance, gradually filling up the whole cell, and pushing the chlorophyll granules to the posterior end of the cell. The smallest paramylum grains are found at the anterior end of the cell in the neighbourhood of the gullet; and they gradually increase in size towards the middle of the cell, where they are much crowded together and nearly all of the same size. Khawkine's explanation of this is that the small size of the grains near the gullet indicates that they begin to form in this region, and that it is probably through the gullet that the substances are absorbed which are necessary for their production.

This is a very interesting observation; but it is obvious that we require further evidence before we can say definitely that liquid nutriment is absorbed wholly, or even partly, through the gullet.

So far, then, all we can say concerning the function of this anterior cavity in the cell of *Euglena* is that it serves for the excretion of liquid from the pulsating vacuole; but that it serves for the ingestion of either solid or liquid food has not been proved. In a later part of this paper I shall refer to its connection with the flagellum.

Structure of the Eye-spot.

It is well known that many motile cells of both animals and plants possess a red pigment-spot which is sharply defined from the protoplasm; and, from its apparent resemblance to the eye of *Cyclops* and Rotifers, was called by Ehrenberg an eye-spot.

Our knowledge of its structure depends upon the researches of Leydig, Kunstler, Klebs (*l. c.*), Schilling, Franzé *, Overton †, Pouchet, Johnson ‡, and others, for an account of which reference

* Franzé, "Zur Morphologie und Physiologie der Stigmata der Mastigophoren." Zeit. f. Wiss. Zool. 1893, vol. lvi. p. 138.

† Overton, "Beitrag zur Kentniss der Gattung *Volvox*." Bot. Centralbl. vol. xxxix. p. 65, 1889.

‡ Johnson, "Observations on the Zoospores of *Draparnaldia*." Bot. Gaz. vol. xviii. p. 294, 1893.

may be made to Zimmermann *, and to the memoirs of Klebs and Franzé.

The eye-spot was at first supposed to be a homogeneous red spot or disc; but Leydig † in 1856 described it in *Euglena* and some other forms as consisting of scarcely measurable, strongly refractive granules; and this has since been confirmed by many other observers.

According to Klebs (*l. c.*) the eye-spot of *Euglena* is a well-characterized body, both in form, inner structure, and chemical behaviour. Its form varies with the species, but is very constant for one and the same species. Its structure is apparently the same in all species, consisting of two substances—a plasmatic ground-mass forming a fine network, and a pigment which occurs in the form of drop-like bodies embedded in it. The presence of a plasmatic substance can only be inferred from the fact that the eye-spot can be caused to expand under pressure or by the action of swelling reagents, which results in the separation of the pigment-drops from one another.

Franzé (*l. c.*) states that, in addition to the pigment, the eye-spot contains one or few to many strongly refractive bodies which are, in *Euglena*, composed of paramylum, grouped in a more or less regular manner, consisting of a central or excentric "crystal body," with smaller and always more numerous "lens bodies" surrounding it. Both the crystal body and the lens bodies serve to concentrate the light, and the pigment not only absorbs light but s also sensitive to light.

I have been quite unable to confirm Franzé's observations either as to the crystal body or the lens bodies, although it is very easy to observe the granular structure described by other observers. I have examined several species of *Euglena* and *Phacus*; I have had *Euglena viridis* under observation in all its stages and under many different conditions of its existence, but I have never seen anything of the nature of paramylum bodies in or on the eye-spot. Nevertheless I have sometimes noticed, when examining the eye-spot with a magnifying-power of 500 or 600 diameters, an appearance something like that figured by Franzé, which seems to be due, however, to the irregular outline of the eye-spot (it is very rarely as regular in outline as Franzé's

* Zimmermann, "Sammel. Referate &c., 10. Der Augenfleck (Stigma)." Beihefte zum Bot. Centralbl. vol. iv. p. 160, 1894.

† Leydig, Lehrbuch der Histologie. (See Franzé, *l. c.*)

figures would indicate), and to the fact that it is curved around the gullet and is sometimes sharply turned in here and there at the edge. The eye-spot has thus not only an irregular contour, but its surface is very uneven, and under a low power with poor definition it may sometimes present an appearance of colourless granules embedded in the pigment. Under a high magnifying-power with good definition, this appearance vanishes completely, and the eye-spot is resolved into the simple granular structure to which reference has already been made.

The pigment granules are brightly refractive and have a very distinct outline. They form a single layer, and in *Euglena viridis* are easily separated from each other, especially when the cells are in the enycsted condition. In some eye-spots the granules are spherical and all of the same size, but in others they are more irregular in shape and of different sizes, and in such cases the eye-spot is more homogeneous in appearance, especially near the middle.

By the action of strong potash solution the eye-spot swells up, and the pigment granules become separated from one another *. The arrangement of the granules is not very definite, but now and then they were found to be grouped in rows, sometimes radiating from the centre (fig. 1, *a*). The number of granules present varies, but not to any great extent: in a fairly large number of cases which I counted, I found that between 30 and 40 granules were the most frequent. If the action of the potash is continued for some time, the eye-spot disintegrates more or less completely into a number of granules (Pl. 32. fig. 1, *d*). Even in the living condition, the eye-spot sometimes breaks up into a number of separate granules which become distributed through the protoplasm. This is frequently found to be the case in cells which have become encysted and surrounded by a thick wall. I have never seen it in elongate motile cells, although I have often noticed in the surrounding protoplasm a number of granules of the same size and colour as those in the eye-spot, and looking as if they had been separated from it. They can be easily distinguished from the rusty-red granules, which appear in the protoplasm as a result of the disintegration of the chlorophyll grains, by their bright red or orange colour and greater refractive power. The nature of this colouring-matter has not been fully

* Klebs, *l. c.*

investigated, but the few observations which have been made seem to show that it is a chlorophyll derivative. According to Cohn, with whom Klebs agrees, the red pigment is hæmatochrome, and stands in genetic relationship to chlorophyll; whilst Rostafinski * regards it as reduced chlorophyll. The rusty-red granules and the pigment of the eye-spot are similar in their behaviour towards alcohol, which in both cases, immediately it comes into contact with them, causes the granules to run together to form a homogeneous red mass or drop of oily substance, from which two colouring-matters at once become separated out—an orange-coloured substance which forms the main mass of the drop, and one or two small bright red droplets in the centre of it. The red colour soon disappears entirely, and the orange colour changes to yellowish green, then to green, and at the same time becomes smaller and smaller until it suddenly disappears, and in its place a small vacuolar-like body is left, surrounded by an irregular ring of some refractive substance.

This reaction, although not conclusive by any means, indicates that the rusty-red granules which are derived from the chlorophyll and the red pigment of the eye-spot have something in common, and supports Cohn's statement that the latter is genetically connected with chlorophyll. It has been suggested that the pigment of the eye-spot is identical with the red colouring-matter, carotin, which occurs in the roots of *Daucus Carota* and in the orange or red chromatophores of many fruits and flowers, from the fact that it shows the characteristic blue colour with sulphuric acid †.

Guignard ‡ has shown that in the Fucaceæ the orange-coloured chromatophores possess the same chemical reactions as the orange-coloured eye-spot; and he further shows that the eye-spot is formed from a colourless chromatophore found on one side of the nucleus, which at first becomes rapidly coloured yellow, then orange.

In *Euglena* the eye-spot is found both in the motile and in the resting cells, and new eye-spots arise by division, as Klebs has also shown. Whether there is any formation of eye-spots *de novo* at any stage in the life-history of *Euglena*, such as occurs in

* Klebs, *l. c.*

† See Zimmermann, Botanical Micro-technique.

‡ Revue Gén. de Bot. i. 1889.

the motile cells of Fucaceæ * and Algæ †, is doubtful. The breaking-up of the eye-spot which has been observed in old encysted cells may simply be a part of the general disintegration of the cell; if not, it would appear that a new formation of the eye-spot must take place on the resumption of the motile condition. This, however, requires further investigation.

Without entering into any further discussion of these facts, it is clear, I think, that the eye-spot is a definite organ of the cell possessing a characteristic, but simple structure; that it is probably a derivative of chlorophyll; that it is present in the motile cells only of those Algæ in which it has been described and is formed *de novo* when they are produced; and that even in *Euglena*, although it is present both in the motile and in the resting condition, it disappears, or becomes broken up, in cells which remain long in the encysted condition. We are probably justified, therefore, in concluding that the eye-spot is an organ of the motile stage of both animal and plant cells, specially connected with their power of movement.

The Flagellum.

The flagellum is an organ of the cell especially connected with its rapid movement through the water. If it is absent, the *Euglena* is only capable of a slow creeping movement which is caused by a peculiar contraction of the body, characteristic of the organism. This contraction appears to originate in the protoplasm, but the different forms assumed are probably to some extent dependent upon the elasticity of the limiting layer or cell-wall; and Khawkine (*l. c.*) suggests that contractile fibres are present—longitudinal ones over the whole of the cell, and transverse ones in the anterior region only. These, however, have not yet been seen, the only indication of any such contractile fibres being the oblique striæ found on the cell-wall; and so far we have no evidence that these play any part in causing the contraction of the cell.

The free movement of the cell through the water is entirely due to the flagellum, which by its rapid motion draws the cell after it, causing it at the same time to rotate around its longitudinal axis. Very little is known of the actual mechanical means by which this is accomplished, but an interesting discussion

* Guignard, *l. c.* † Overton, *l. c.*, and Johnson, *l. c.*

of the question will be found in the first volume of the 'Traité de Zoologie Concrète' * by Delage and Hérouard.

It is usually stated that the flagellum arises either on the dorsal wall of the gullet or from the protoplasm at its base. This, however, is not correct. The flagellum not only does not terminate in the gullet, but actually passes into the excretory reservoir, with which, as I have shown, the gullet is in open communication, and it becomes attached to its dorsal wall, or occasionally to its ventral, by a bifurcate base (Pl. 32. figs. 2 to 8). This bifurcation extends as far as the anterior edge of the eye-spot, at which point the single whip-like portion of the flagellum begins (fig. 2). This mode of attachment of the flagellum has not, so far as I am aware, been observed before; it probably serves to give it a much firmer support, and may be of mechanical advantage to it in its movement.

In addition to this bifurcation, we find another interesting structure connected with the flagellum. On one of the branches of the bifurcate base there occurs an oval swelling or enlargement, immediately in front of the eye-spot, and just below the point where the bifurcation begins (Pl. 32. figs. 2, 8). It is in close contact with the eye-spot when the cell is in the elongate motile condition; but it is not actually in organic connection with it, as shown (Pl. 32. figs. 7, 8), and in numerous cases which were observed of *Euglena*-cells in the resting condition, it was completely separated from it (fig. 9). In some cases, this enlargement appears in the position indicated in figs. 3 to 6, just above, or at, the point of bifurcation of the flagellum; but from a careful examination of numerous specimens in which this appearance was seen, it seems to me that it is an effect due to the second branch of the bifurcation passing below the enlargement, and so becoming hidden from view (compare figs. 7 and 8).

Although both the bifurcation and the enlargement are visible under favourable conditions, in the living cell, it is necessary in order to see them clearly to resort to the use of reagents. The best reagent for the purpose is a 1 per cent solution of osmic acid. This kills motile *Euglena*-cells at once, and leaves them in an expanded condition with their flagella distinctly visible. They should remain in this solution from three to forty-eight hours or more, and may be examined in dilute glycerine. In order to preserve them for future investigation, they may be kept in a

* La Cellule et les Protozoaires, p. 305.

50 per cent. solution of alcohol. Dilute glycerine is the best mounting medium, but fairly satisfactory preparations may be made either in glycerine-jelly or canada balsm.

In good preparations the contents of the cell appear black, the eye-spot black and sharply defined, and the flagellum with its enlargement dark grey.

I have occasionally succeeded in staining the flagellum and enlargement light green in a mixture of methyl-green and fuchsin. Fig. 2 is from a preparation made in this way and mounted in dilute glycerine.

The Effect of Light on Euglena.

In common with many other unicellular motile organisms which contain chlorophyll, *Euglena* is extremely sensitive to light. Our knowledge of the phenomena is mainly due to the investigations of Stahl *, Strasburger †, and Engelmann ‡. They find that in general the cells are attracted by a light of moderate intensity and repelled by an intense light; but that the degree of sensitiveness which they exhibit varies considerably even in individuals of the same species. Further, both Klebs and Strasburger have shown that they do not lose their sensitiveness to light either in the dark or at a higher temperature; and Englemann has also shown that this power is independent to a high degree of a variation in the oxygen pressure.

The effects produced by the action of light upon the motile cells of *Euglena* are very striking. They are strongly attracted by a bright light such as that of a gas-flame or incandescent burner focussed, by means of a substage condenser, upon a microscope-slide on which a drop of water containing *Euglenæ* has been placed. Such a spot of light will attract, in the space of about one minute, the majority of the cells in the field of the microscope, as seen by a one-inch objective; and in two minutes only very few will be found outside the light area. If a large number of cells are present, they will form a seething mass in

* "Ueber den Einfluss von Richtung und Stärke der Beleuchtung auf einige Bewegungserscheinungen im Pflanzenreiche." Bot. Zeitung, 1883.

† "Wirkung des Lichtes und der Wärme auf Schwärmsporen." Jena. Zeitschr. xii., 1878.

‡ "Ueber Licht und Farbenperception niederster Organismen." Pflüger's Archiv, Bd. 29, 1882 (see J. R. M. S. 1883, p. 81).

the light space, perhaps two or three layers deep, all of them with the anterior end pointing downwards towards the source of light. On turning down the condenser so as to get the light spread evenly over the whole field of the microscope, they begin at once to move away rapidly in all directions, and in one or two minutes are found in all parts of the field. If the light is again focussed upon a small portion of the field, the *Euglenæ* turn round suddenly and make for it; and a steady stream of them will be seen moving up to the light from all parts of the field. When the light space is shifted from place to place in the field of the microscope, the *Euglenæ* follow it; and if the mirror be moved backwards and forwards in such a manner that the light space is made to pass continually from one side of the field to the other, the *Euglenæ* arrange themselves all across the field in the area marked out by the passage to and fro of the light space. The rapidity with which they move up to the light space is much greater when there is no diaphragm over the condenser than when one is present. The diffuse light outside the bright spot apparently guides them.

It is interesting to watch the movements of the *Euglenæ* in the light space. The majority of them move about freely, but appear to be unable to get out; for directly the anterior end of an individual passes into the shade, it turns round completely and goes back again into the light. Some of them, however, may pass completely into the shade, and may even swim for some distance into the dark part of the field before turning round; others again do not turn round completely at once, but move round the edge of the light space in a tangential direction for some distance before passing in again.

Some of the cells which are strongly attracted to the light space move straight across it, and pass out again on the opposite side into the dark part of the field. After traversing this for some distance, they turn round and repeat the performance; and they may do this three or four times in succession before they come to remain in the light space.

If a drop of water containing motile *Euglenæ* be placed on a glass slip and exposed to bright sunlight, the *Euglenæ* move to that side which is farthest from the sun. If the slip is turned round, they at once begin to move towards the opposite side, that which is now farthest from the sun; and in the space of three minutes a large number of *Euglenæ* are able to pass from one side to the other, across a drop of water a quarter of an inch in diameter. If in their passage across, the sun becomes obscured by clouds, or if a sheet of note-paper is placed between the sun and the drop of water, they stop at once, and either remain moving about in the middle of the drop or go back again to the side nearest the source of light.

If they are kept in bright sunlight for any length of time, they come to rest and round themselves off; and if they remain in a good light for some days, the cells gradually become encysted. The effect of a strong light is always to produce encystment.

In a light of moderate intensity, the cells may remain motile for a very long time; but they always become rounded off at night, and may then lose their flagella and undergo division into two. Until they lose their flagella, however, they may be always brought into the motile condition again by exposing them to the light of a lamp or gas. This shows, as Klebs* has pointed out, that they do not lose their sensitiveness even in the dark so long as they can move.

These experiments are sufficient to show that *Euglena* possesses a very definite light-perception. Engelmann has also shown that it is the colourless anterior end of the cell which is sensitive to light, and it is only when this comes into contact with light or shadow, that the cell reacts to the light by altering the direction of its movements. If the shadow falls upon the posterior chlorophyll-containing end of the cell, there is no reaction. He has further shown that *Euglena* prefers the blue portion of the spectrum, the following being the percentage distribution of a typical case:—

Red to Orange	1·4 per cent.
Orange to Green	0 ,, ,,
Green........	11·2 ,, ,,
Green to Blue	70·4 ,, ,,
Blue to Indigo	16·8 ,, ,,
Indigo to Violet	2·1 ,, ,,

It has also been shown that swarmspores are more sensitive to the blue rays of the spectrum than to other parts.

According to Franzé (*l. c.*) and Wildeman † motile *Euglenæ* are sensitive to heat, but they do not respond to it either so quickly or so intensely as to light.

* *Loc. cit.* p. 263.

† "Sur le thermotaxisme des Euglènes," Bull. Soc. Micros. Belg. 1894.

To determine the effect of heat, Franzé placed *Euglenæ* in a thin glass tube which was closed at both ends, and kept warm at one end by allowing a stream of hot water to flow over it. The tube was kept in the dark. At a temperature of 55° C. the *Euglenæ* became immobile from the effects of heat, but at a lower temperature, 30°–40° C., they mostly approached the source of heat.

Wildeman obtained somewhat similar results. He placed *Euglenæ* in a tube with wet sand, in order to avoid convection-currents. It was then placed in the dark in a horizontal position, and warmed at one end, and he found that the *Euglenæ* accumulated at the warmer end of the tube at a temperature of 30° C. On exposing tubes to light as well as heat, he found a considerable modification in the effects produced. If they were placed at right angles to the rays of light, the *Euglenæ* avoided the warm end of the tube. If they were placed in the same direction as the rays of light they moved towards the light, even when the opposite end of the tube was heated.

My own experiments confirm in a general way the results obtained by these observers; but the *Euglenæ* are so slightly sensitive to heat as compared with light, that the subject requires further investigation before any very definite conclusions can be arrived at.

The Function of the Eye-spot.

It is commonly stated that the eye-spot is a light-perceiving organ. This does not imply that it possesses an actual visual function, but simply that it is connected in some way with those changes in the direction of the movements of the cell which are due to light. There is no direct proof of this, but we have a sufficient amount of indirect evidence to show that the statement is probably a correct one.

Ehrenberg regarded the pigment-spot in *Euglena* as a light-perceiving organ on account of its general resemblance to the eyes of Rotifers and *Cyclops*; and it has since been shown that it resembles them in some respects both in structure and in its behaviour towards solvents and other reagents, such as iodine and sulphuric acid *.

Further, in all those chlorophyll-containing unicellular organisms which are very sensitive to light and capable, by means

* Klebs, *loc. cit.*

of their flagella or cilia, of responding quickly to changes in its direction or intensity, an eye-spot is present. Those motile cells which do not possess an eye-spot are either not sensitive to light at all, or only to a slight extent. Even in the zoospores of *Chytridium*, which according to Strasburger * are sensitive to light, it is interesting to note that at the base of the cilium there is a conspicuous orange-coloured oil-globule, which may act in the same way as an eye-spot.

Again, Engelmann has shown for *Euglena* and Strasburger for swarmspores of Algæ, that the rays of light which are most active in their influence upon the movements of these organisms are found in the region of the blue portion of the spectrum, and these are just the rays which, as indicated by the colour of the eye-spot, are absorbed by it. We conclude from this, therefore, that it is the light absorbed by the eye-spot, and not that transmitted through it, which is concerned in these movements.

Finally, it has been shown by Engelmann that it is the colourless anterior end of *Euglena* that is sensitive to light. "Hence, in this case, a certain part of the body functions to a certain extent as an eye"†. In this colourless anterior end of the cell, both the eye-spot and the apparatus which directly causes the movement of the cell—the flagellum—are placed.

Having thus briefly stated the evidence we possess in favour of the conclusion that the pigment-spot of *Euglena* is a definite light-perceiving organ, we must now attempt some explanation of the way in which the light acts.

It is obvious that, whatever may be the action of light, the movements of the cell, as well as any change in direction of its movements, are dependent directly upon the flagellum. Without it, as we have seen, the cell is only capable of a very slow contractile movement, of its body from place to place. It is evident therefore that those rays of light which are capable of exerting a material influence upon the movements of the cell can only do so by controlling or modifying in some way the mechanism by means of which the flagellum is caused to move. Now we have already seen that the flagellum and eye-spot are closely related to each other. The flagellum arises near the eye-spot and, on its

* Jena. Zeitschr. xii. 1878, p. 568.

† Hertwig, 'The Cell,' p. 100.

way to the exterior, passes quite close to it. Moreover it possesses an enlargement on one of its basal supports which is placed immediately in front of, and in close contact with, the inner concave surface of the eye-spot.

The presence of this enlargement of the flagellum in such a position at once suggests the simple explanation that the light-rays which are absorbed by the eye-spot cause a stimulation of the enlargement in some way ; and this stimulation reacting upon the flagellum causes its movements to become modified, and so results in a change in the direction of the movement of the cell.

We have thus a combination of two structures, which may, if my explanation be correct, be regarded as an extremely simple form of eye, consisting of a specialized portion of the cell (*protoplasm* ?) possessing great sensitiveness to light-rays of a particular kind, and a pigment-spot, as a light-absorbing organ, in close contact with it.

Whether it is the light absorbed by the pigment-spot which actually effects the change in the movements of the flagellum; or whether the pigment-spot simply prevents these rays from reaching one side of the enlargement, whilst the other side is left freely exposed to them, thus producing a difference of intensity on the two sides of the enlargement, it is at present impossible to say. We know that protoplasm itself is sensitive to light, and responds to it without the intervention of any such structure as a pigment-spot. It may be therefore that the arrangement just described in *Euglena* is one by which the active rays are, under certain conditions, cut off on one side of the enlargement, while the other is left exposed to them. This would result in a definite unequal illumination of the sensitive portion of the cell ; and consequently the organism would tend to move into such a position that it would be more or less equally illuminated all round, and would be brought ultimately into the light-ray.

I put forward this explanation tentatively, as the only one which appears to me at present to afford an adequate interpretation of the facts observed. It is evident, however, that it is a question which demands a more extended and laborious investigation than I have so far been able to undertake, before any very definite conclusions can be formulated.

Summary.

(1) The structure of the eye-spot in *Euglena viridis*, *E. Ehrenbergii*, and *Phacus pyriforme* is not as Franzé has described it. The so-called "crystal body" and "lens bodies" were not found in any of the individuals of the species examined by me. As earlier observers have stated, the eye-spot consists simply of a mass of pigment granules arranged in a single layer, and probably embedded in a protoplasmic framework, but the latter could not be seen, and its presence was only inferred from the fact that the pigment granules must be held together in some way, that the eye-spot as a whole is capable of expansion, and that the pigment granules can be separated from each other.

(2) The gullet at the anterior end of the body does not end blindly, as was formerly supposed, near the principal vacuole, but actually opens into it, thus forming one continuous cavity open to the exterior.

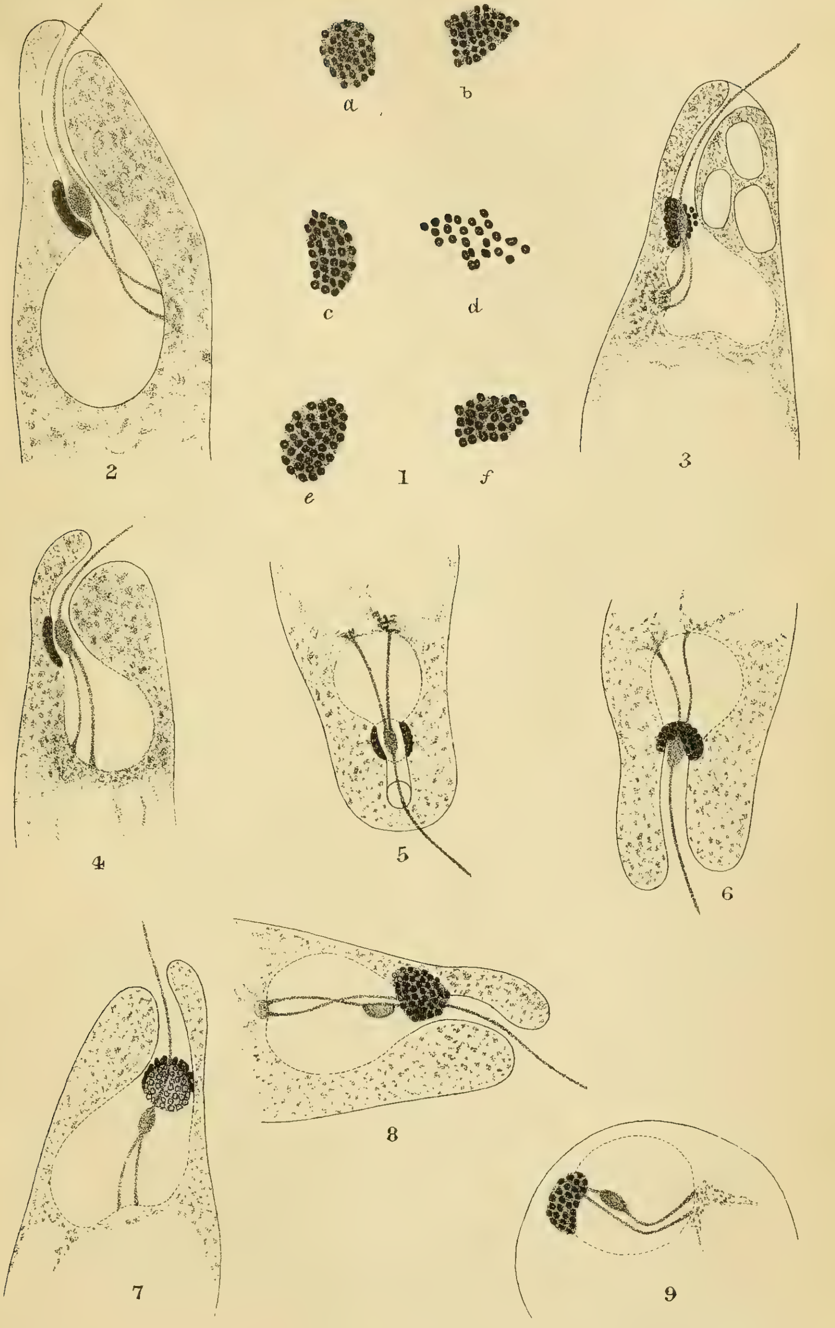
(3) The eye-spot is in close contact with the gullet at the point where it opens into the excretory reservoir, and curves around it slightly.

(4) The flagellum is not attached to the wall of the gullet, but arises from near the posterior or basal side of the excretory reservoir, to which it is attached by a bifurcate base. The bifurcation begins at about the level of the eye-spot, and in this region an enlargement of one of the basal supports takes place. This, which is oval in shape, is in close contact with the eye-spot, on its inner concave side. It is in the same position as that described by Franzé for his "crystal body," and may have been mistaken for this by him.

(5) An explanation of this structure suggested by the author is that the light which is absorbed by the eye-spot in some way stimulates this enlargement, which in turn reacts upon the flagellum and causes its movements to become modified. This is supported by the facts which are stated in the three following paragraphs.

(6) The rays of light which are absorbed by the eye-spot—those in the region of the blue of the spectrum—are the same as those which are active in modifying the direction of movement of the *Euglena*-cell.

(7) These rays can only influence the movements of the cell by acting upon or controlling in some way the mechanism by which the flagellum moves.



H. Wager del.
M.P. Parker lith.

Geo. West & Sons imp.

EUGLENA VIRIDIS.

(8) All those chlorophyll-containing motile cells which are very sensitive to light contain an eye-spot. Many other organisms which do not possess an eye-spot are sensitive to light, but not to so high a degree.

(9) If the explanation given in par. 5 is correct, we have in *Euglena* an extremely simple form of light-organ, consisting of a specialized sensitive portion of the cell—the enlargement on the flagellum—and a light-absorbing pigment-spot in close contact with it.

EXPLANATION OF PLATE 32.

The figures have been drawn, unless otherwise stated, with the aid of the Camera lucida, and the $\frac{1}{12}$th oil-immersion of Leitz or the 2 mm. 1·4 apert. apochromatic objective of Zeiss and ocular 18.

Fig. 1. Eye-spots from different individuals, showing the arrangement of the pigment-granules.

Fig. 2. Side view of the anterior end of a motile cell, showing the flagellum and its enlargement in front of the eye-spot. This specimen was stained in methyl-green and fuchsin for two hours, then washed in water and mounted in dilute glycerine. The whole of the red colour was washed out, the flagellum and its enlargement were stained light green; the eye-spot was left brown.

Fig. 3. This shows the attachment of the bifurcate base of the flagellum to the dorsal wall of the excretory reservoir. The protoplasm formed a slight projection at the point of attachment, and was slightly more hyaline or refractive than the rest.

Fig. 4. The flagellum is attached to the excretory reservoir nearer the base than in fig. 3, and each of its arms is slightly expanded at the base. The enlargement appears just at the place where the bifurcation begins.

Fig. 5. A view of the anterior end of a cell as seen from below.

Fig. 6. A slightly oblique view of the anterior end of a cell seen from above.

Figs. 7 & 8. Two views of the same cell, showing the enlargement at some distance from the eye-spot.

Fig. 9. A freehand sketch of a portion of a cell in the resting condition. The excretory reservoir, the eye-spot, and the bifurcate base of the flagellum and its enlargement are shown.